

Movie 1: The initial contacts between Tregs and BMDCs are stable

CFSE-labeled Tregs (green) were introduced to peptide-pulsed BMDCs that were pre-seeded onto coverslips and imaged once per minute. CFSE fluorescence (green) is overlaid on DIC images. The majority of Treg-BMDC conjugates were stable over the length of the experiment. This movie is related to Fig. 1B.

Movie 2: Tregs form motile contacts with BMDCs 24 hrs after the initial Treg-BMDC interactions

Unlabeled Tregs were added to peptide-pulsed BMDCs at a 5:1 ratio and incubated for 24 hrs. Fresh CFSE-labeled Tregs were then added to the wells and imaged once per minute. CFSE fluorescence (green) is overlaid on DIC images. Both the unlabeled and CFSE-labeled Tregs primarily made motile contacts with the BMDCs. Still images from this movie are shown in Fig. 1D.

Movie 3: Tregs form motile contacts with supported planar bilayers containing only pMHC and ICAM-1

Tregs were introduced to supported planar bilayers containing pMHC and ICAM-1 and imaged once per minute. ICAM-1-Cy5 fluorescence is shown. The Tregs were highly motile over the entire length of the experiment. This movie is related to Fig. 3A.

Movie 4: Tregs form motile contacts with supported planar bilayers containing pMHC, ICAM-1 and 40 molecules/ μm^2 CD80

Tregs were introduced to supported planar bilayers containing pMHC, ICAM-1 and 40 molecules/ μm^2 CD80 and imaged once per minute. ICAM-1-Cy5 fluorescence is

shown. Most of the cells were highly motile over the entire length of the experiment.

This movie is related to Fig. 3B.

Movie 5: Tregs are significantly less motile when interacting with supported planar bilayers containing pMHC, ICAM-1 and 200 molecules/ μm^2 CD80

Tregs were introduced to supported planar bilayers containing pMHC, ICAM-1 and 200 molecules/ μm^2 CD80 and imaged once per minute. ICAM-1-Cy5 fluorescence is shown. The cells displayed significantly decreased motility compared to Tregs interacting with supported planar bilayers with 0 or 40 molecules/ μm^2 CD80. This movie is related to Fig. 3C.